FUNCTIONAL ANALYSIS OF SWIM-BLADDER MUSCLES ENGAGED IN SOUND PRODUCTION OF THE TOADFISH

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ABSTRACT

A functional analysis of the striated swim-bladder muscles engaged in the sound production of the toadfish has been performed by simultaneous recording of muscle action potentials, mechanical effects, and sound. Experiments with electrical nerve stimulation were made on excised bladder, while decerebrate preparations were used for studies of reflex activation of bladders *in situ*. The muscle twitch in response to a single maximal nerve volley was found to be very fast. The average contraction time was 5 msec. with a range from 3 to 8 msec., the relaxation being somewhat slower. The analysis of muscle action potentials with surface electrodes showed that the activity of the muscle fibers running transversely to the long axis of the muscle was well synchronized both during artificial and reflex activation. With inserted metal microelectrodes monophasic potentials of 0.4 msec. rise time and 1.2 to 1.5 msec. total duration were recorded. The interval between peak of action potential and onset of contraction was only 0.5 msec. Microphonic recordings of the characteristic sound effect accompanying each contraction showed a high amplitude diphasic deflection during the early part of the contraction. During relaxation a similar but smaller deflection of opposite phase could sometimes be distinguished above the noise level. The output from the microphone was interpreted as a higher order derivative function of the muscle displacement. This interpretation was supported by complementary experiments on muscle sound in mammalian muscle. The dependence of the sound effects on the rate of muscle contraction was demonstrated by changing the temperature of the preparation and, in addition, by a special series of experiments with repeated stimulation at short intervals. Results obtained by varying the pressure within the bladder provided further evidence for the view that the sound initiated in the muscle is reinforced by bladder resonance. Analysis of spontaneous grunts confirmed the finding of a predominant sound frequency of about 100 per second, which was also found in reflexly evoked grunts. During these, muscle action potentials of the same rate as the dominant sound frequency were recorded, the activity being synchronous in the muscles on both sides. Some factors possibly contributing to rapid contraction are discussed.

INTRODUCTION

In many teleosts the swim-bladder is engaged in the production of sounds which may serve as a means of communication (9, 14). In some species the sound is produced by expulsion of gas bubbles through the pneumatic duct. In other species, which have a closed swim-bladder, a different mechanism exists comprised of special striated muscles which initiate vibrations in the bladder wall. In drumfishes these muscles have one attachment on the body wall and one on the bladder, and in other sound-producing fishes, *e.g.*, the sea robin and toadfish, the attachments are entirely on the bladder wall. The role of these so-called intrinsic muscles in the mechanism of sound production has been studied experimentally by various workers (6, 7, 23). Tower (23) came to

the conclusion that in the toadfish the synchronized contraction of the striated muscles on both sides caused the tense walls of the swim-bladder to vibrate, thus producing the sound. More recently, the sound of the toadfish has been analyzed by Fish *et al.* (9, 10) who found the principal frequency of the typical "grunts" to be about 100 per second, while the "boat whistle" sound, produced in the breeding season only, had a strong fundamental tone in the vicinity of 325 per second.

In the present investigation the functional properties of the intrinsic swim-bladder muscles of the toadfish have been studied with electrophysiological techniques. The principal finding of the analysis, a short account of which has been published previously (22), is that the contraction rate of these muscles is extremely fast and that this is essential for the production of the characteristic sounds.

METHODS

In most experiments the swim-bladder was dissected out, placed on a rubber block and kept in moist air (room temperature 21°C.). A few experiments were performed on the bladder *in situ* after removal of the intestines (decerebrate preparation with spontaneous respiration).

Stimulating electrodes were placed on the free end of the muscle nerve which had been cut close to its exit from the spinal cord. Muscle action potentials were recorded by bipolar silver-wire electrodes on the muscle surface or by inserted steel microelectrodes a few μ in tip diameter.

Muscle contraction was measured by an RCA 5734 transducer with the insulated stylus placed directly against the muscle surface, or by recording the pressure changes within the bladder via an inserted syringe needle connected to a "Techtrinol" capacitance manometer (15) which accurately reproduced changes up to 1,000 cycles per second.

In most positions of the stylus the recorded movement (upward deflections in all records except Fig. 6 *B* and *C*) represented an inward displacement of the muscle surface. The concomitant increase in pressure was recorded as downward or upward deflections in the records.

For sound recording, an electrodynamic pressure-operated microphone (60 to 10,000 cycles) was used which ordinarily was placed as close to the lateral side of the bladder as possible without making direct contact. The microphone was connected to one of the D.C. amplifiers of the Dumont cathode ray oscilloscope. In some experiments on natural sound production the sound was recorded on tape ("Revere tape recorder T-1100" microphone of similar frequency range as above), from which the recordings could later be played back into the oscilloscope and photographed on moving film. In spite of the somewhat varying dimensions and properties of the available microphones, "Electrovoice model 1647," "Economy and King model de luxe, Radio Shack," and the one belonging to the tape recorder, the wave form of the microphone output was practically identical. The polarity of the microphone output was not determined; in most of the records the initial phase of the recorded sound wave was recorded downward during the contraction and upward during the relaxation phase.

A comparative series of experiments on mammalian muscle was performed in Stockholm, mainly on the tibialis anticus and gastrocnemius of cat and rabbit. The hindleg was rigidly mounted in a stand and the muscle tendon connected to a barium titanate transducer (or one of capacitance type); alternatively the lateral displacement of the muscle belly was recorded. Electrical differentiation of these effects was performed by the use of time constants of suitable values in the amplifier circuit. For sound recording, microphones of similar properties as above were used.

RESULTS

I. Analysis of Muscle Contraction

Anatomical Data: The intrinsic muscles of the toadfish's swim-bladder form oval structures of 40 to 50 mm. length covering the lateral sides of the bladder (20). The muscle fibers run transversely to the long axis of the muscle and are about 10 mm. in length. The motor nerve enters the muscle at its cranial end and runs close to the bladder wall, giving off fine side branches during the first two-thirds of its course, after which the main stem divides in a fan-like way innervating the caudal part of the muscle.

Nerve Conduction: The nerve action potential, recorded over conduction distances up to 40 mm., showed a smooth contour even at supramaximal stimulation, indicating uniform fiber sizes (Fig. 1); histological sections showed a strikingly uniform fiber spectrum with an average fiber diameter of 10 μ. Determinations of conduction speed gave values between 25 and 30 m. per second.

Contraction Curve of Single Twitch: In view of the unique anatomy of the swim-bladder muscles, a conventional recording of tension or shortening of the muscle fibers is difficult. Attempts to suspend a muscle dissected out from the bladder were not

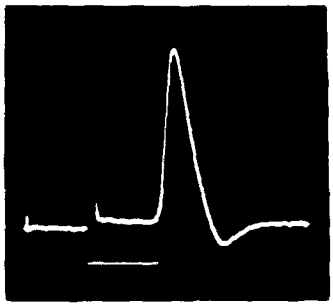

FIGURE 1

Action potential of nerve to swim-bladder muscle. Supramaximal stimulation. Conduction distance 25 mm. Time bar 1 msec.

successful. Because the muscle is shaped to fit and to be expanded on the surface of a sphere, it proved to be difficult to stretch all fibers effectively by pulling the longitudinal muscle edges apart. The muscle was therefore preferably studied *in situ* under its normal working conditions. Recording of the movement of the muscle by placing the transducer stylus against its surface may not give the same picture of the course of contraction as that obtained, *e.g.*, by measuring the shortening of the muscle. However, on the basis of data from other muscles, it can be assumed that the moments of onset, peak and termination of contraction, as determined by the recording of surface movement, correspond on the whole to those which might have been obtained by conventional recording. In favor of this conclusion is also the fact that similar results are obtained when using the increase of the pressure within the bladder as an index, as will be exemplified in the following.

The swim-bladder muscle responds to a single nerve volley with an extremely rapid twitch, some examples of which are given in Fig. 2. The shape of the curve varies somewhat, which may partly be ascribed to the recording method; usually its contraction is distinctly faster than relaxation, as illustrated in *A*. The contraction time of 5 msec. in *C* represents the average value, the range being from 3 to 4 msec. to 7 to 8 msec., as exemplified in *A* and *B*. The rate of contraction and also of relaxation is, to a certain extent, dependent on the pressure within the bladder (*cf.* Section II). The relaxation phase often shows an "overshoot" (*C*) which may be a first phase in a damped oscillation (*D*). These phenomena are interpreted as being rebound effects due to the elastic properties of the bladder after its compression; they are reflected also in the pressure recording (Fig. 3 *C* and *D*).

Normally the pressure in the excised bladder amounts to 40 to 50 mm. water (23). At the peak of contraction the pressure is increased by 8 to 15 mm.; when both muscles are activated this value is doubled. As shown in Fig. 3 the time course of the pressure change is similar to that of the muscle contraction. There is usually a delay of at least 1 msec. in the onset of the pressure curve in relation to that of the contraction curve (*B*). This may be due to a certain inertia in the pressure recording system.

It is instructive at this point to compare the time course of the contraction and relaxation phases with corresponding data from muscles of another cold blooded animal, the frog sartorius and extensor digitorum longus. Typical values for the contraction time at 20°C. are 30 to 40 msec. (5, 19), and the total time for attaining complete relaxation may amount to 100 msec. The toadfish's swim-bladder muscle is thus up to ten times faster. Some of the factors accounting for this fast contraction will be analyzed in the following.

Muscle Action Potential and Its Time Relation to Contraction: When analyzing the action potential of the swim-bladder muscle, it is essential to keep in mind that the spread of activity in the longitudinal direction of the muscle is not, as in *e.g.* sartorius,

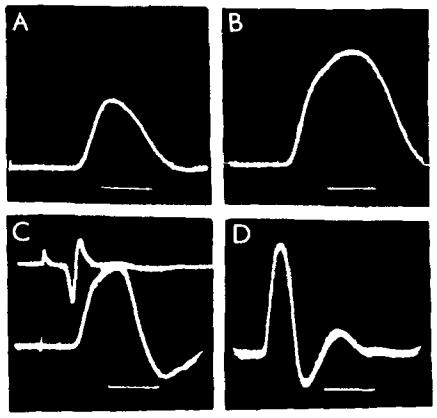

FIGURE 2

Contraction curves of three different swim-bladder muscles (*A*, *B* and *C–D*) obtained by recording displacement of muscle surface (*cf.* "Methods"). Upper beam in *C* muscle action potential recorded with surface electrodes. Time bar in *A–C* 5 msec; in *D* 10 msec.

due to the depolarization wave but is a result of a successive recruitment of transversely arranged muscle fibers as the nerve impulses travel along the motor nerve. Since the depolarization waves in the individual muscle fibers run in crosswise direction, the compound muscle action potential recorded with surface electrodes involves complex volume conduction phenomena, which makes a potential analysis rather intricate. The present analysis has therefore been limited to certain time factors which are relevant to the main scope of this investigation, the contraction velocity.

The potentials recorded with one electrode at each end of the muscle are often polyphasic, as shown in Fig. 4 *A*, and have a duration of 2.5 to 3 msec., representing the total time of electrical muscle activation. When the surface electrodes are closer together, either in transverse or longitudinal arrangement, potentials of diphasic shape and slightly shorter duration are obtained (Figs. 2 *C*, 3 *A*, 5). Determinations at fast sweep speed of the difference in onset of depolarization at the cranial and caudal ends of the muscle give values of about 0.3 to 0.5 msec. (Fig. 4 *B*). With inserted microelectrodes, which allow more accurate readings, similar differences have been obtained. The observed values are smaller than those which would be expected from a nerve conduction at 30 m. per second over a muscle length of 30 to 45 mm., *i.e.*, 1.0 to 1.5 msec. The difference between observed and calculated values may be explained by unselective recording, which has to be taken into account as long as intracellular recording is not being used. However, it is also possible that some special mechanism for synchronization is present. Preliminary observations suggest that the nerve branches, which seem to be differently arranged in the proximal and distal end of the muscle, deserve a closer study, especially with respect to the diameter of the fibers. A high degree of synchronization between the units involved is obviously a primary requisite for attaining a short contraction time for the whole muscle.

It proved difficult to determine the conduction rate of the depolarization wave along short muscle fibers like these; intracellular recording seems necessary for obtaining reliable values and, besides, data about the localization and histological structure of the motor end-plates also seem to be indispensable for such an analysis. However, independently of the absolute value of the conduction rate of the muscle spike, the small conduction distance within the short muscle fibers will obviously be one favorable factor in attaining a short activation time for the individual muscle fiber.

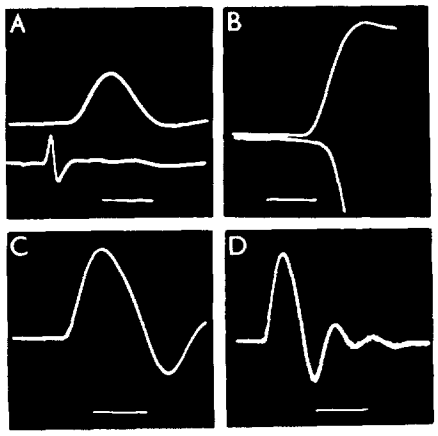

FIGURE 3

Contraction curves of three different swim-bladder muscles (*A*, *B*, and *C–D*) obtained by recording pressure changes in the bladder. Increase in pressure recorded as upward deflection except in *B*. Lower beam in *A*, muscle action potential recorded with surface electrodes; upper beam in *B*, surface movement of muscle. Time bar in *A–C* 5 msec., in *D* 10 msec.

The potentials recorded by means of inserted metal microelectrodes are of shorter duration, about 1.2 to 1.5 msec., indicating a more selective recording. Sometimes the potential shows an all-or-none behavior, suggesting single unit recording, but this may also be due to the difficulty of grading the stimulus effect in the motor nerve with its uniform fiber spectrum and evidently narrow range of threshold values. The monophasic shape of the potential suggests that muscle fibers have been punctured. It is impossible to say how similar the recorded potential is to that obtained by proper penetration with capillary electrodes, but one might assume that the true intracellular potential is not likely to be of longer duration. This assumption allows one to compare the time characteristics of the potential recorded with metal microelectrodes from the swim-bladder muscle fibers with those of the intracellular potential recorded from the frog's foot muscle. For the former, the rise time to the peak, 0.4 msec., and the total duration, 1.2 msec., are about half the corresponding values for the frog muscle of 0.7 and 3 msec. (5). Although no definite conclusion

can be drawn from such comparisons between fibers of different length and diameter (5), it is very likely that the rapid de- and repolarization processes of the swim-bladder muscle potential are an expression of specific membrane properties.

In Fig. 4 *C*, simultaneously with the action potential, a recording was made of the onset of contraction. This is seen to take place within the period of electrical activation. In order to get a more exact determination of the time relationship between the two events, some special experiments were performed in which microelectrodes were used for recording of the muscle potential and the stylus of the transducer placed close to the recording site and arranged for maximal mechanical sensitivity to detect the earliest change in muscle tension. A typical result from such an experiment is illustrated in Fig. 4 *D*, where the first contraction effect is recorded during the falling phase of the action potential. It might be argued that, with the arrangement used for recording movement, remote parts of the muscle may transfer their mechanical effects to the stylus and that the error thus introduced may be considerable if the propagation in the muscle is slow. However, one might then expect great variations of the onset of muscle contraction in relation to the action potential at the arbitrarily chosen recording sites, but this was not the case.

The interval between the peak of action potential and the onset of the mechanical effect is only 0.5 msec. which again is much shorter than the corresponding value of 4.3 msec. for the frog's foot muscle (5). (Even the preceding relaxation phase observed in the frog has a latency of 2 msec. Such an initial relaxation, if existing in the swim-bladder muscle, is not likely to appear in these experiments in view of the comparatively insensitive recording method used.)

No attempts have been made to measure the minimum time for nerve-muscle transmission, since this is considered to be of small analytical interest as long as morphological data of end-plate organization are missing. However, the interval between the peak of nerve impulse recorded at the entry into the muscle and the gross muscle action potential has been measured in some experiments, the average value being 1.5 msec.

Summation of Contractile Effects: Application of a

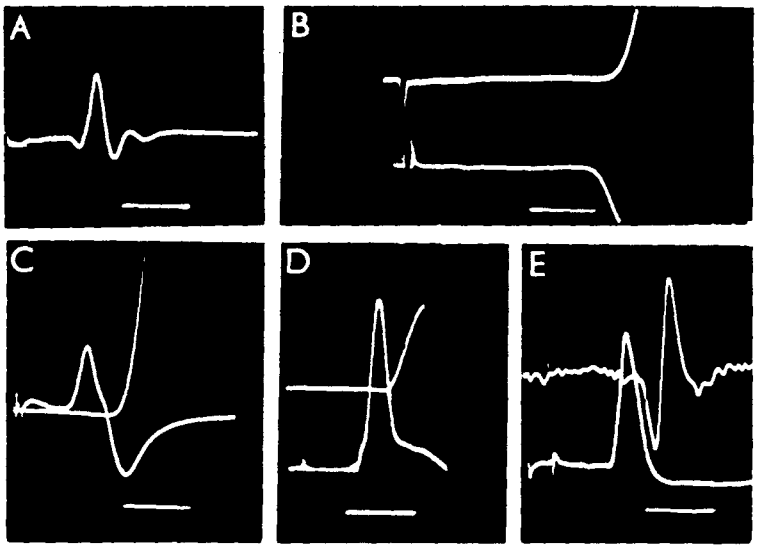

FIGURE 4

A, muscle action potential recorded with surface electrodes between both ends of muscle. *B*, fast sweep record showing slight difference in onset of muscle action potential recorded simultaneously at the cranial (lower beam) and caudal end of muscle (upper beam). *C*, muscle action potential recorded in the same way as in *A* (upper beam) and onset of contraction (lower beam). *D*, muscle action potential recorded with metal microelectrode (lower beam) and onset of contraction (upper beam). *E*, muscle action potential recorded with metal microelectrode (lower beam) and sound effect (upper beam). Time bar in *A*, *C-E* 2.5 msec.; in *B* 1 msec.

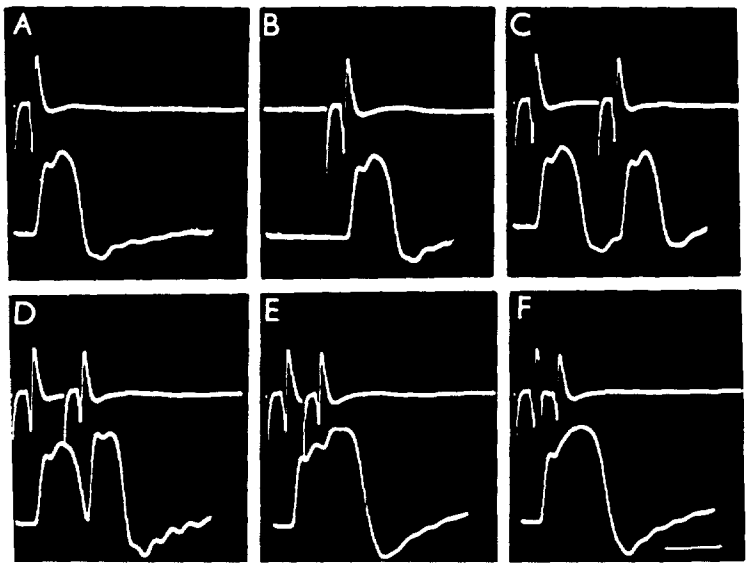

FIGURE 5

Summation of contractile effects by application of two successive stimuli. Upper beam muscle action potential. Lower beam contraction curve. Baseline oscillations at about 300 per second probably induced by the recording system whose resonance frequency when unloaded was 500 per second. *A* and *B*, first and second stimulus applied separately. Stimulus interval in *C* 14 msec., *D* 9 msec., *E* 6 msec., and *F* 4 msec. Time bar 10 msec.

second stimulus to the nerve, before relaxation is complete, results in summation of the contractile effects, as shown in Fig. 5. The total contraction-relaxation time for this muscle was 10 msec. Stimulation at intervals above this value results in separate contractions of equal sizes (Fig. 5 *C*). In Fig. 5 *D*, at a stimulus interval of 9 msec., the second effect is slightly superimposed, and in Fig. 5 *E* with an interval of about 6 msec. the two effects have fused into one contraction of higher amplitude and longer duration, which is maintained also at an interval of 4 msec. (Fig. 5 *F*). The second muscle action potential undergoes no significant reduction in amplitude at intervals above 3 msec. The variations in rate of second contraction which may occur will be commented on in Section II.

II. Analysis of Sound Production

In freshly excised swim-bladder preparations each muscle twitch is accompanied by a characteristic "pop" sound, the loudness of which increases with the stimulus strength up to a maximum at full contraction. When after some 20 to 30 minutes, the preparation becomes less excitable and the muscle contraction weaker and slower, the sound becomes less distinct and may finally disappear. Even these observations suggest that the rate of contraction is an essential factor for production of the sound.

Microphone Recording: With the microphone type available for these experiments, sound effects may, under favorable conditions, be recorded during both the contraction and the relaxation phases. The first diphasic deflection seen in the microphone record during the early part of the contraction phase is the largest and most constant (Fig. 6 *A*). The following waves may be more or less masked by the noise level which necessarily was rather high under the provisional conditions in the laboratory, which was far from soundproof; however, a distinct deflection can often be distinguished during the relaxation phase, as exemplified in Fig. 6 *B*. It is also diphasic but the reverse of that seen during contraction.

The microphone output has tentatively been interpreted as a higher-order derivative function of the muscle displacement, caused by the radiation of the sound as well as by the microphone itself. Differentiation of enlarged tracings of some

contraction curves has been performed by graphical methods, and although such a procedure is crude, in some cases there was found to be a good correspondence between the second derivative of the contraction curve and the microphone output (Fig. 12 *A*). Unfortunately, the possibility of performing electrical differentiation of the transducer output for direct comparison with the microphone output was not used during the actual experiments. This procedure has later been applied in a comparative series of experiments on mammalian muscle, the results of which give further support to the above interpretation (*cf.* Section III).

Of the microphone output, only the initial deflection, which can always be recorded with sufficient amplitude in preparations in good condition, will be subjected to a closer analysis. The magnitude of this deflection seemed to be in good agreement with the loudness of the sound perceived and, judging from the subjective impression of sounds in experiments with stimulation at short intervals (*cf.* below), this deflection apparently corresponds to the essential component in audible sound, although other components represented by the later low-amplitude oscillations in the microphone record may modulate the sound impression.

The initial deflection, being a derivative function of the contraction phase, constitutes a sensitive index of the onset of contraction which may be difficult to determine from the transducer record of muscle displacement. Thus, with moderate amplification of the transducer output, as in Fig. 6 *A* and *B*, the sound appears to precede the onset of contraction. In fact, great care had to be taken in the application of the transducer and a high amplification was required to make the contraction curve take off simultaneously with the first phase of the microphone output, as in Fig. 6 *C*.

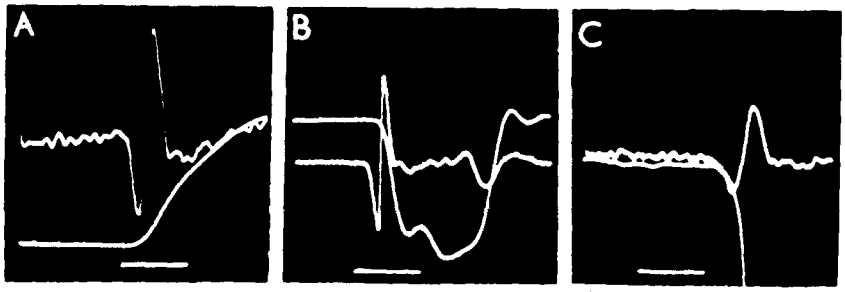

FIGURE 6

Sound effects recorded simultaneously with contraction curves. *A*, high amplitude effect during initial part of contraction. *B*, effects in relation to whole contraction curve (recorded downward). *C* (same muscle as in *B*), initial effect related to onset of contraction curve recorded with higher gain and faster sweep. Time bar in *A* and *C* 2.5 msec., in *B* 5 msec.

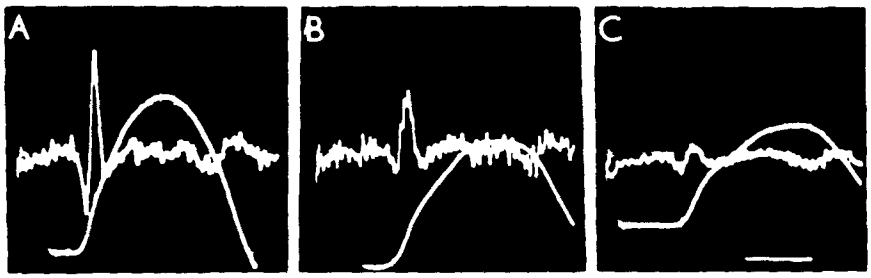

FIGURE 7

Influence of lowering of temperature on sound effects (upper beam) and contraction curve (lower beam). *A*, before; *B* and *C*, after irrigation of the nonmuscular part of the bladder with ice water, causing a successive lowering of the temperature of the muscle below room temperature (fall in temperature not measured). Time bar 5 msec.

This convenient measure of initial mechanical changes in the muscle has been used, *e.g.*, in determinations of the time relation between muscle action potential and onset of contraction, and Fig. 4 *E* shows an example of the minimum interval which may be obtained by simultaneous recordings of the electric and acoustic effects.

Experimentally induced variations in the rate of rise of contraction result in changes of the microphone output of a kind to be expected from the mathematical relationship postulated above. Thus, Fig. 7 shows the decrease in amplitude of the diphasic deflection when the contraction is slowed by lowering the muscle temperature. Similar changes in sound effects are also induced during successive blocking by curare or by a decrease of stimulus strength.

When the pressure within the bladder is decreased below normal, the sound becomes weaker and its character changes so that it appears more dull. Conversely the sound becomes stronger and sharper when the pressure is increased within certain limits. These changes in the perceived sounds correspond to changes in amplitude of the recorded sound effects (Fig. 8). Two factors could be involved in these phenomena. First, since the muscle fibers are suspended on the elastic bladder wall, they undergo changes in initial tension which will affect the contraction curve. Secondly, the change in tension of the bladder wall is likely to influence the resonance properties of the bladder.

The complex acoustic phenomena involved in such secondary resonance effects are beyond the scope of this paper. It is important to point out, however, that sounds can be heard and recorded also when the bladder is nearly completely collapsed (Fig. 8 *A*) which indicates that the sound is primarily set up in the muscle and amplified by the resonance of the inflated bladder. The primary importance of the rate of muscle contraction is also evidenced by the casual observation that if the muscle contraction at normal bladder pressure is too slow and weak to produce any audible sound, it cannot be elicited only by increasing the pressure in the bladder.

The records in Fig. 9, from an experiment where two successive stimuli were applied to the nerve, are illustrative of the relationship between the rate of contraction, or relaxation, and the amplitude of the recorded sound effects. Records *A* and *B* (Fig. 9) show the similar responses to the two stimuli applied separately. (There are small

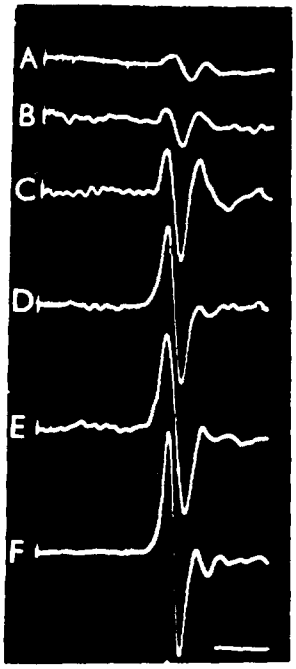

FIGURE 8

Effect of varying bladder pressure on initial sound effects (maximal contractions). *A*, bladder partly collapsed. *B–F*, pressure in mm. water: *B* 7, *C* 10, *D* 40 = normal, *E* 65 and *F* 75. Time bar 2.5 msec.

humps in the contraction curve in this experiment, recorded as pressure variations, which are not likely to be recording artifacts but which may actually represent a stepwise contraction since they are reflected also by minimally perceptible deflections in the microphone curve. However, these will be disregarded in the following.) It appears that in addition to the initial high amplitude deflection during the early contraction there is a rather well marked effect during relaxation.

In record *C* (Fig. 9) the second contraction starts in the overshooting relaxation phase of the first contraction, and it can be clearly seen that the deflections in the microphone record, especially during contraction but also during relaxation, are of higher amplitude after the second than after the first stimulus. A closer examination of the contraction curves reveals that there is a corresponding difference in slopes of the initial parts of the contraction and relaxation phases, the rate of change being faster in the second than in the first curve.

In the following records (*D*—*F*, Fig. 9) where the stimulus interval is successively diminished, summation of the contractile effects occurs. In *D*, at an interval of 8 msec., the onset of the second contraction is clearly marked as a stepwise increase in the contractile effect, although somewhat less steep than the onset of contraction after the first stimulus. The second sound effect is therefore also smaller. In the next record, *E*, the additional contraction effect due to the second stimulus is still sufficient to produce a distinct deflection in the microphone recording, but in the last record, *F*, in this series the two contractile effects have fused so that the second sound effect during the contraction phase is hardly recognizable on the baseline. The sounds recorded during relaxation phases also become successively smaller, the rate of change apparently becoming slower with diminishing amplitude of contraction. By listening to the sounds at the same time as observing the effects recorded on the screen, it was evident that the dominant sound impression corresponds to the initial deflection in the microphone output. It was also noted that at intervals of about 10 msec. the sound was most similar to the natural grunt.

For comparison, a series of experiments was also performed in which the second stimulus was applied to the nerve of the other muscle (records *G*—*I*, Fig. 9). It appears that the sound effects then occur independently of each other even at

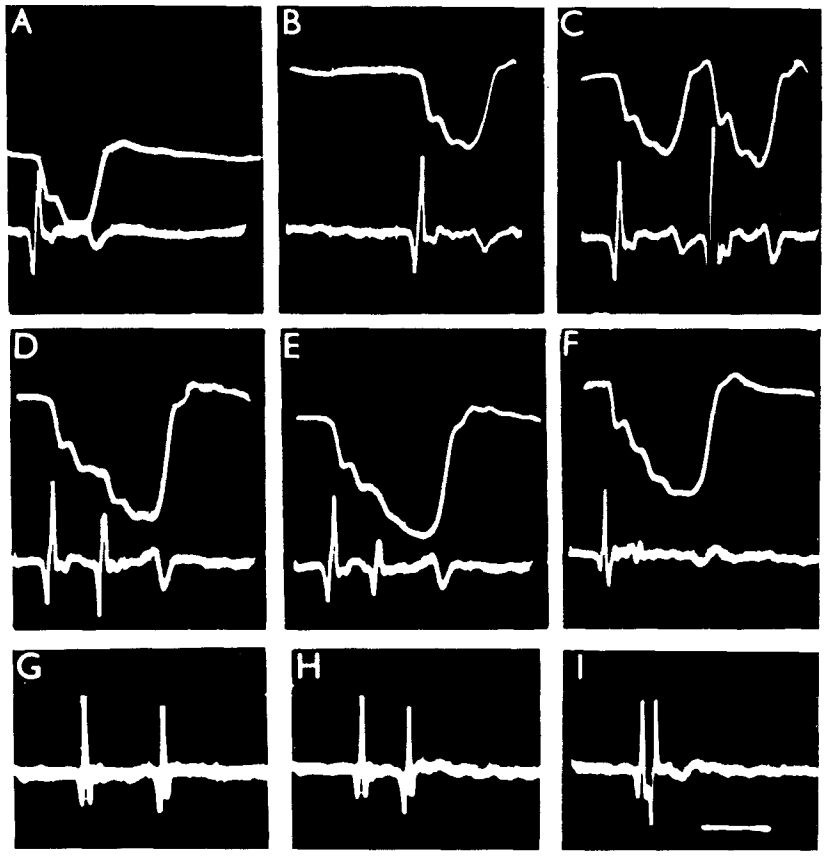

FIGURE 9

Sound effects (lower tracings) occurring when two successive stimuli are applied to the nerve of one muscle (*A-F*), and to each of the nerves to the two swim-bladder muscles (*G-I*). *A* and *B*, responses to first and second stimulus applied separately. Stimulus intervals in msec.: in *C* 15, *D* 8, *E* 7, and *F* 5; *G* 12, *H* 7, and *I* 2. Upper tracings in *A-F* muscle contraction curves recorded as pressure changes within the bladder (pressure increase downward). Time bar 10 msec.

short intervals, as was to be expected when the sound primarily originates in the muscles and the role of the bladder as a resonator is only secondary.

Continued frequent stimulation of a muscle over longer periods results in sound effects of the type illustrated in Fig. 10. In records *A* and *B* (left and right muscle of the same bladder) the stimulus frequency has been 50 per second, corresponding to a stimulus interval of 20 msec. At this rate there is thus a contraction-free interval between the stimuli. The initial effects during the rise of each contraction are recognized as high amplitude diphasic deflections of short duration. In between these there are seen slower waves of varying amplitudes which are interpreted as summation phenomena. In addition to small amplitude responses during relaxation there may be more or less distinct after-oscillations, and these may summate, particularly at frequencies close to the bladder's natural resonance frequency.

At a frequency of 50 per second, stimulation can usually be maintained over many seconds without any sign of fatigue (Fig. 10 *B*), but sometimes even at this frequency, depending on the state of the muscle, there is a successive decline in amplitude of the initial output from the microphone, indicating a diminishing contraction rate with each consecutive stimulus (Fig. 10 *A*). The fatigue phenomena are more pronounced at higher rates. Thus, the same muscle as in Fig. 10 *B*, when stimulated at 100 per second, shows a distinct decline of the sound effects (Fig. 10 *D*).

The subjective impression of the sound produced at stimulus frequencies of 100 per second and higher is that of a musical tone of corresponding pitch, with a certain "roundness" evidently due to a modulation by overtones, which fades out more or less quickly depending on the stimulus rate.

It is obvious that with alternating stimulation

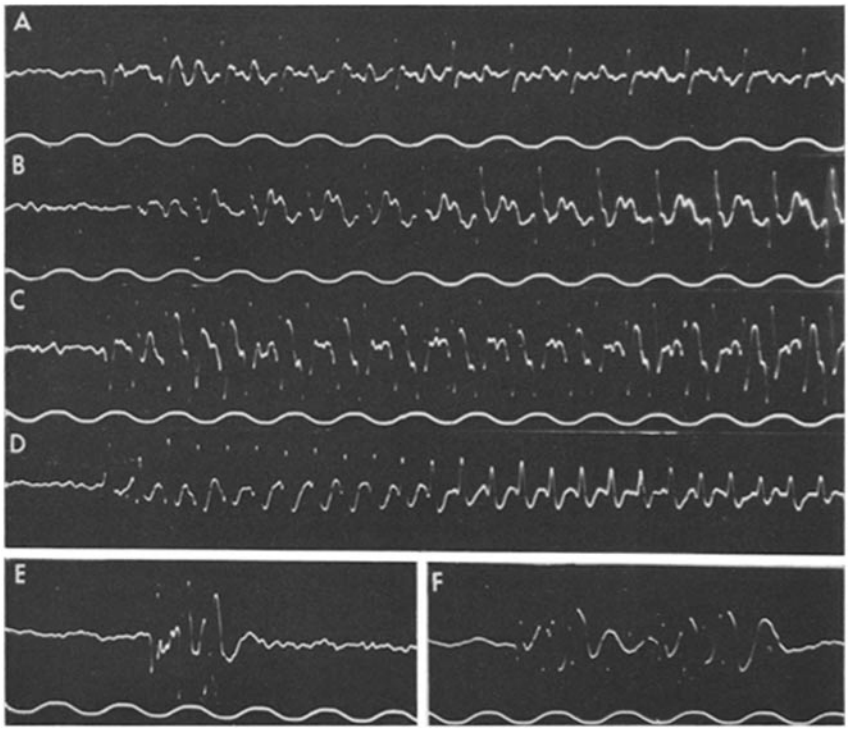

FIGURE 10

Sound recordings from swim-bladder during repetitive stimulation. *A*, stimulation of left muscle at (about) 50 per second. *B*, stimulation of right muscle of same bladder at 50 per second. *C*, simultaneous stimulation of both muscles at 50 per second with 10 msec. phase shift. *D*, stimulation of right muscle at 100 per second. *E* and *F*, sound recordings of spontaneous grunts. Time marking 50 cycles per sec.

of the two muscles in the same bladder, *e.g.*, at 50 per second with a phase shift of 10 msec., a tone of twice this frequency is obtained, which is of course maintained without decrease in amplitude much longer than that produced by a single muscle excited at 100 per second (Fig. 10 *C*). Rather prolonged tones of 200 to 300 per second can be obtained by such alternating stimulation.

Natural Sound Production: The microphone recordings of spontaneous grunts, produced by the fish when taken out of the water, show the same diphasic deflections of short duration as after artificial nerve stimulation (Fig. 10 *E*). The grunts, usually built up by intermittent sounds (Fig. 10 *F*), last from $\frac{1}{10}$ to $\frac{1}{2}$ second. Each high amplitude deflection is followed by a slower wave similar to those occurring during repetitive nerve stimulation. The interval between each sequence is about 10 msec. which agrees well with the results from the frequency analysis of the toadfish sounds by Fish (9).

For the recording of muscle action potentials

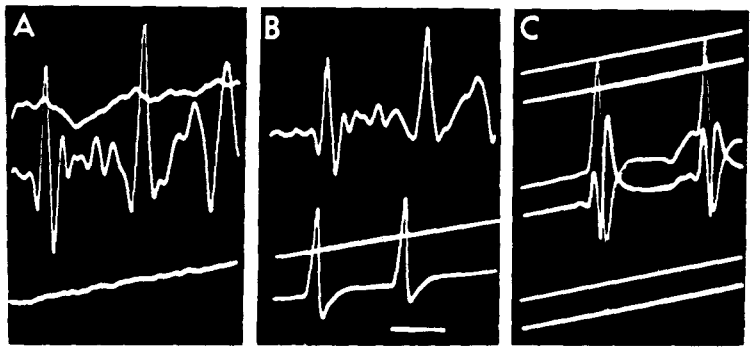

FIGURE 11

A and *B*, sound recording of reflexly evoked grunts. Lower beam in *B*, action potentials recorded with surface electrodes. *C*, action potentials from the two muscles of the same bladder showing synchronous discharge. Time bar 5 msec.

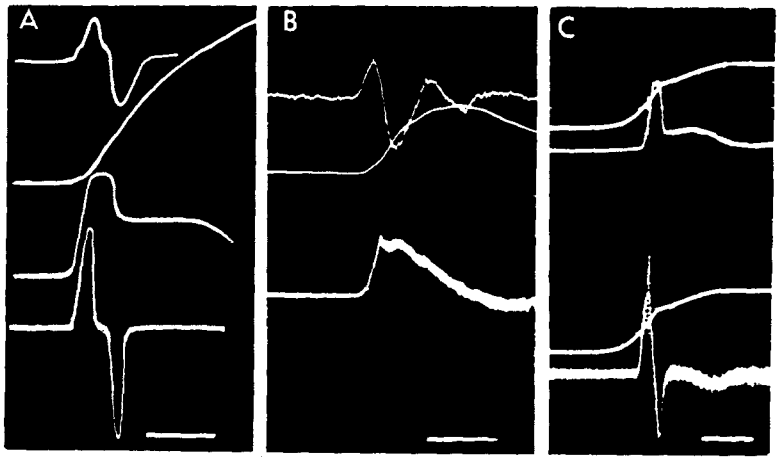

FIGURE 12

Comparison of microphone output and derivatives of contraction curves. *A*, swim-bladder muscle. From above: tracings of microphone record, contraction curve, first and second derivatives obtained by graphical methods. Time bar 2.5 msec. *B*, tibialis anticus of cat. From above: sound recording, contraction curve and its first derivative obtained by electrical differentiation. Time bar 25 msec. *C*, tibialis anticus of cat. From above: contraction curve and its first derivative; contraction curve and its second derivative obtained by electrical differentiation. Time bar 10 msec.

decerebrate preparations were used in which the bladder was exposed by laparotomy and partial extirpation of the intestines. If such preparations were breathing spontaneously and were kept with the gills submerged in sea water, it was possible by various methods of afferent stimulation, *e.g.*, pulling the mesentery, to evoke reflex grunts. These sound like the voluntary ones and show similar microphone output patterns, as appears from Fig. 11 *A*. The action potentials, led off with surface electrodes, occur simultaneously with the high amplitude deflections in the sound record and are of the same configuration and duration as those following electrical nerve stimulation, indicating a well synchronized muscle activity (Fig. 11 *B*). As far as could be judged from a few experiments with simultaneous recording from the two muscles, the discharges occur simultaneously on both sides (Fig. 11 *C*).

III. Comparative Experiments on Mammalian Muscle

In order to throw some more light on the mechanisms of sound production during muscle contraction, a preliminary series of experiments on the cat's tibialis anticus muscle were also performed, in which simultaneous recordings of muscle action potentials, sound, and contraction effects were made. The contraction effect was recorded in the conventional way at the tendon. In some experiments the lateral displacement of the muscle belly was also measured using a capacitance transducer without contact on the muscle surface. The contraction peaks were found to coincide in the two curves which were thus, to a certain extent, comparable.

A condenser microphone of the same frequency characteristics as that available at Woods Hole, when placed over the muscle belly, recorded a diphasic wave early during the contraction phase (Fig. 12 *B*). This corresponds with the results on the swim-bladder muscles, although the time constants of the two muscles are very different, as seen by comparing Fig. 12 *A* and *B*, in which there is a tenfold difference in time scale. By electrical methods the first or second derivatives of the transducer potential from the muscle contraction were obtained and recorded on the second beam of the scope. As appears from Fig. 12 *B* and *C* the microphone record of the sound of mammalian muscle can also be interpreted as higher-order derivative functions of muscle contraction.

DISCUSSION

The small contraction and relaxation times found for the swim-bladder muscle place it among the fastest muscles in the animal kingdom. As described in Prosser's (19) comparative data on time constants of muscle, only the fastest muscles of warm-blooded animals, such as the eye muscles of the cat, show contraction time values of the same order of magnitude, 7.5 msec.

Some of the factors possibly contributing to the fast contraction, such as short fibers in transverse arrangement, synchronous activation and a brief duration of the muscle spike, have been discussed in connection with the previous analysis. The fact that both ends of the muscle are free to move symmetrically may also contribute to a quick contraction. This would be further facilitated if there are motor end-plates at each end of a single muscle fiber, as has been described for certain fish muscles (2).

However, it is obvious that the rate of contraction depends ultimately on the properties of the contractile elements and their activation through the excitation-contraction coupling. The role of the sarcoplasmic reticulum for inward conduction of excitation, first suggested by Retzius (21), has recently been considered by Bennett, Porter and Palade, and others (3, 13, 17, 18) who have made detailed electron-microscopic studies of these structures referred to as "triads." Peachey and Porter (16) proposed the attractive theory that the speed of contraction might be related to the degree of development of the reticulum. In view of this hypothesis, Fawcett's findings (8) of an exceptional regularity and abundance of triads in the toadfish's swim-bladder muscle are of great interest and very suggestive. It might also be assumed that, in addition to an effective system for excitation-contraction coupling, the properties of the contractile elements themselves may be of great importance for the achievement of high contraction speed.

From Section II it has appeared that the characteristic sound accompanying each muscle twitch can be attributed to the fast rate of contraction, the dominant sound component being initiated in the earliest part of the contraction curve. In the transition from the relaxed state to

contraction the greatest acceleration is to be expected and this was confirmed by graphical differentiation of the contraction curve. The results from the comparative series of experiments on mammalian muscle gave further support to the view that the sound is primarily due to the pressure wave set up by the displacement of the muscle during contraction. This is the most obvious explanation but, keeping in mind that intramuscular pressure variations not directly associated with the contractile effects may also occur during muscle excitation, it is obvious that contributing factors for sound production in muscle may be revealed. The primary role of the muscles in the mechanism of sound production of the swim-bladder was early recognized by Dufossé (6) who also pointed out the close relation to the phenomena of muscle sound studied, *e.g.*, in mammals.

The shortest duration, 10 msec., observed for the total contraction-relaxation period permits a repetition of a complete contraction cycle at a rate sufficiently high to account for the fundamental sound frequency, 100 per second, of the spontaneous grunts. However, as was shown by application of two stimuli at short intervals, a distinct sound effect could be produced even when the muscle had not been allowed to relax completely, provided that the rate of the additional contraction was fast enough. That muscle sounds may thus be produced in states of subtetanic contraction is not unique for the swim-bladder muscle but is a phenomenon well known from classical studies of sounds in mammalian muscle (4), which have shown that with electrical stimulation muscle tones of the same frequency as the muscle action potential can be produced up to the frequency for complete mechanical fusion. During voluntary contraction of musculus orbicularis oculi in man, sounds from single motor units at a discharge rate of 45 per second have been recorded by Gordon and Holbourn (11).

The other type of sound, similar to the blast of a boat whistle (9), produced by the toadfish in the breeding season, is of such a high fundamental frequency, 325 per second, that it is not likely that it could be produced by subtetanic oscillations of the muscle, unless the properties of the muscle are fundamentally changed by hormonal factors in the spawning period. Another explanation has been advanced in Section II, namely, alternating contraction of the two muscles which, when artificial stimulation was used, could be shown to double the tone frequency. In the grunts (the only type of sounds that could be produced reflexly or spontaneously during the time of the experimentation), only synchronous activation of the two muscles was recorded, but the assumption that the toadfish would be capable alternately to contract the swim-bladder muscles on each side may not be too speculative since such contractions occur in swimming movements. Resonance phenomena may also be involved in the production of higher tone frequencies but, as pointed out earlier, the analysis of such acoustic phenomena are outside the scope of this paper.

In a study of the cranioclavicular muscles engaged in the sound production of the sculpin, a similar relationship between frequency of sound and muscle action potentials has been found (1).

Finally, it is of comparative physiological interest that rapid muscle contractions are involved in a mechanism of sound production serving purposes of communication, particularly since a theory has been proposed (12) implying that oscillatory contractions of the muscle fibers of the vocal cords may play a role in human phonation.

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